SEEDING AN ADHERENT CELL BIOREACTOR WITH NON-ADHERENT CELLS INCREASES SEEDING DENSITY LIMIT AND REDUCES REQUIRED EXPANSION TIME

RELATED APPLICATIONS

[0001] This application is a Divisional of co-pending U.S. Ser. No. 15/306,830 filed 26 Oct. 2016, which is a National Stage entry of and asserts priority to PCT Application Serial No. PCT/US2015/046927 filed 26 Aug. 2015, which in turn asserts priority from Great Britain patent filing serial no. GB14/17042.7 filed 25 Sep. 2014, the contents of which are here incorporated by reference.

GOVERNMENT INTEREST

[0002] None

FIELD OF THE INVENTION

[0003] We have found a counter-intuitive way to improve the commercial-scale production of recombinant biological products in adherent-cell bioreactors. Our approach reduces the risk of cell culture contamination, increases total yield and reduces the delay between seeding and harvest, thus minimizing expression product degradation.

BACKGROUND OF THE INVENTION

[0004] To date, there is only one approved gene therapy product, Glybera®, which contains an adeno-associated virus for the treatment of a rare familial lipoprotein lipase deficiency (Salmon et al. 2014). Nevertheless, a broad spectrum of gene therapy applications show potential for future development and commercialization. Reaching patient doses of 10^{12}-10^{13} viral particles (vp) for smaller-scale clinical trials has proved a difficult obstacle so far, and large scale manufacturing of viral vectors for commercial supply (i.e., $\geq 10^{16}$ vp) remains a challenge. The manufacturing challenge was recently reviewed by (Vellinga et al. 2014).

[0005] The range of producer cell lines is wide. Cells for in vitro culture come in two general types: cells adapted to live adhered to a solid substrate, and cells adapted to live in suspension in a liquid medium. Adherent cell lines include e.g., HEK293 (and derivatives like 293T and 293FT cells), CEF, MDBK, A549 and Vero. These have been used for the production of different biological products such as adenovirus, MVA, bovine herpes virus, AAV or influenza virus (Knowles et al. 2013).

[0006] Not all cells can be efficiently grown in suspension, and in addition, some cells in adherent conditions have shown higher specific vector productivity (Iyer et al. 1999). Therefore the efficient scaling up of the adherent production systems is required. The traditional way to culture adherent cells is in a flask, and these can vary in type (e.g. T-flasks, roller bottles, Cell Stacks, Hyper Stacks or Cell Factories). Upscaled production in flasks is limited by the production space required, it is impractical to handle and the multiple units make it difficult for monitor/control culture conditions.

[0007] There are different kinds of carriers or substrates available for use in adherent-adapted cell culture bioreactors. For example bead-type micro-carriers (e.g., Cytodex, GE healthcare) float freely in suspension in media. Matrix type carriers (e.g., Fibra-cell disks, Eppendorf) consist of polyester fibers, optionally held within a cage (for example, of polypropylene) to immobilize the fibers. We here use the terms "carrier" and "substrate" to mean any solid-state material used to provide a surface onto which adherent cells may adhere in culture.

[0008] Bead-type microcarriers have been tried in bioreactors (Wu et al. 2002), the micro-carrier providing a substrate for adherent-cell growth while being dispersed in the cell culture medium. These systems, however, have not proven easy to handle, and do not ensure homogenous growth. Another limitation has been the expansion of large cell mass on the static vessels with limited scalability. They also need labor-consuming operations for their separation from the vector later in the process (Dormond et al. 2009).

[0009] Challenges in Productivity are

[0010] 1. Lack of controlled automatic production systems

[0011] 2. Low single cell productivity which increases the cell number/volume

[0012] 3. Lack of large scale bioreactor systems to support high productivity

[0013] 4. Lack of disposable bioreactor systems to avoid demanding cleaning and sterilization steps in GMP

[0014] 5. Challenges in functional feeding strategies (e.g. perfusion membrane blockage during the suspension cell culture)

[0015] 6. Cell mass (cell density) is not enough to support high yield production (cell expansion step)

[0016] 7. Low total yield per batch

[0017] 8. Aggregation/precipitation during the downstream process

[0018] The use of packed-fiber bed adherent cell culture bioreactors have provided optional controlled, perfusable system with low shear stress (Meuwly et al. 2007). The iCELLis® bioreactor (commercially available from Artelis S.A (Paris France)/Advanced Technology Materials Inc. (Brussels, Belgium)/Pall Corporation (Fall River, Mass.) represents a fixed-bed bioreactor developed for scalable adherent VERvert Origin (VERO) cell cultures. iCELLis is a single-use compact fixed-bed bioreactor with a carrier made of polyester fibers. The commercial iCELLis system varies between 0.53 and 500 m^2, having two different compaction densities of the carriers (Knowles et al. 2013). Such bioreactors have been used for different kinds of adherent cell process systems. These include the use of different cell lines, such as HEK293, CEF, MDBK, A549 and Vero, and for the production of different biological products, such as adenovirus, MVA, bovine herpes virus, AAV or influenza virus (Knowles et al. 2013) (Lennaertz et al. 2013).

[0019] Even though large capacity adherent cell culture systems provide a theoretically-attractive production system, the expansion of the cell mass prior to initial loading for a large scale adherent culture bioreactor can become a major obstacle during the process. It has been calculated that 15 times 1700 cm^2 roller bottles or 30 times 850 cm^2 roller bottles was needed for the loading of Vero cells into an adherent culture bioreactor providing 500 m^2 of adherent cell culture area (Knowles et al. 2013). However, the seeding density is cell line-dependent and needs to be developed for each individual process. When they were using 293T cells, the loading density was 25 times higher (80 000 cells/cm^2) (Lennaertz et al. 2013) which means that to a inoculate an